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**13. ABSTRACT** *(Maximum 200 Words)*
Two Smithsonian organizations, the Monitoring and Assessment of Biodiversity Program (SI/MAB) and the Center for Tropical Forest Science (CTFS), continue ongoing research projects in Western and Central Africa. Previous funding led to the development of eleven SI/MAB 1-ha research plots and the 50-ha CTFS Korup Forest Dynamics Plot. These research plots seek to better understand forest dynamics and tropical forest ecosystems through analysis of forest biology and spatial distribution, and species identification. Current research efforts in the Korup Forest Dynamics Plot focus on data management and correction, as well as complementary research projects analyzing forest structure and composition, demography, and phenology. Community outreach and training continues in the project through two SI/MAB training courses in long-term biodiversity monitoring (adaptive management) and one CTFS training course in field botany. Two graduate research projects, one focusing on rural socio-economic conditions and the other on medicinal plant screening, proceed in the Korup Forest Dynamics Plot, supplementing the extensive forest dynamics database.

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## INTRODUCTION:

The African ICBG, a project jointly sponsored by the U.S. National Institutes of Health, the National Science Foundation and the U.S. Department of Agriculture has the main focal point of establishing an integrated program for the discovery of biologically active plants for drug development and biodiversity conservation, while ensuring that source countries derive maximum benefits for their biological resources and their intellectual contribution. BDCP facilitates the drug discovery component of the ICBG and therefore serves as a link between the drug discovery part of the program, the biodiversity conservation component and the economic development projects.
This Associate Program has the following as its main objectives:

1 To conduct ethnobiological inventory of plants in the selected study areas;
2. To guide the ICBG in its plant selection and collection strategies for drug discovery. Samples identified from ethnobiological inventory will be collected from biodiversity plots and from wild flora and screened for possible biological activity.
3. To perform phytochemistry and preliminary bioassays on selected plants.
4. To perform plant extraction, bioassay-guided isolation, structural elucidation with research training and infrastructure development being important components of each operation.
5. To maintain and expand the database on African medicinal plants, which includes information on local names, traditional, uses, floristic data, possible constituents, conservation status, agronomic data and economic value. This involved the re-structuring and expansion of the existing AfricMed database to include data from other Associate Programs. This Computerized Information System of African Medicinal Plants (CISAMAP) will be linked to other regional databases.
6. To conduct a socio-economic value assessment of the biological resources in the study area which seeks to::
    I) highlight the non-commercial value of forest products within the cultural/ religious context;
    II) quantify the economic value of biological resources for comparison with other land use options;
    III) place in priority order the production and marketing of biological resources in local markets to provide income for local residents;
    IV) provide baseline agronomic data for the formulation of a sustainable management plan for the forest resources; and
    V) train local natural resource managers and users at the National and Community levels to conduct economic and market research which will integrate the connection between conservation and development. The ICBG may organize rural farmers to cultivate, in fallow areas, certain plants of potential therapeutic value;
7. Assist in capacity building of West African scientists in the areas of ethnobiology, inventory and research management. Formal training will be organized in ethnobiological methods and field taxonomy and economic value assessment for local communities.

## 2. BODY:

### DRUG DISCOVERY

## 2.1 Antimalarial activity

A total of 120 plant samples comprising of 11 pure compounds, 47 purified fractions and 62 extracts were submitted for *in vitro* activity against two strains of *Plasmodium falciparum*. Nine out of the eleven pure compounds belong to the anthraquinone class while the 62 extracts were new plant samples.

The isolation and elucidation of the chemical structures of different active components in some of these antimalarial plants has been reported earlier. In this current period we looked at the structure activity relationship (SAR) of some of the lead compounds. An example of such SAR was based on the anthraquinones, which occur widely in plants but in only a few animals. In our earlier bioassay-guided chromatographic fractionation of extracts of *Morinda lucida*, anthraquinones were shown to have some inhibitory effects on the growth *of Plasmodium falciparium in vitro*. We then considered anthraquinone derivatives as potential lead compounds in the development of new antimalarials. A literature search revealed a modest antiplasmodial activity of some anthraquinones. InterCEDD in collaboration with Dr. Rogelio Pereda-Miranda of the Universidad Nacional de Mexico submitted 9 anthraquinone compounds for *in vitro* testing against *P. falciparium*. As illustrated in Table 1 and Fig 1 (structures of anthraquinones), the two most active anthraquinones (iv & v) had activity against both chloroquine-susceptible (D6) and chloroquine-resistant strains (W2) of *P. falciparium* with IC50 values of 1177.4 and 1248.5ng/ml respectively. An interesting feature is that the compounds are equipotent against chloroquine-susceptible and chloroquine-resistant strains of *P. falciparium*.

In continuation of our investigation on *Pachypodanthium staudtii*, the six compounds previously isolated from *P. staudtii* (PS1-6) with PS1 showing the highest activity ($IC_{50}$ 3367.64 and 986.58ng/ml for D6 and W2 respectively), further bioassay-directed fractionation of the same extract at the School of Pharmacy, University of Pittsburg yielded more potent fractions whose structures are being determined.(Table 2). The $IC_{50}$ values of the most active fractions are 11.3 and 83.1ng/ml for D6 and W2 respectively. Bioassay-guided fractionation of *Pachypodanthium staudtii* and *Cleistopholis paten* yielded forty-seven column fractions which were submitted for *in vitro* testing. Out of which 94% of the chromatographic fractions demonstrated significant antimalarial activity which was higher than that observed with the crude extracts. Table 2 shows the results of the action of purified fractions of *P. staudtii* and *C. patens* against *Plasmodium falciparum*. As shown in Table 3 more than six fractions of *P. staudtii* were equipotent against chloroquine-susceptible and chloroquine-resistant strains of *P. falciparium*. In fact some of them were highly active more particularly against the chloroquine-resistant strains of *P. falciparium* while others showed moderate activity. The hits in this fractionation are more than 94%. Therefore promising antimalarial lead structures could be developed are expected from this plant species.

In summary, the most active plants in our priority list are seeds of *Picralima nitida*, stem barks of *Araliopsis tabouensis, Pachypodanthium staudtii, Enantia chlorantha, Ancistrocladus barteri and* roots of *Uvaria chamae*. Others include *Morinda lucida Spathodea campanulata, Synclisia scarbrida, Cryptolepis sanguinolenta, Glossocalyx brevipes, Cleistopholis patens, Leidobotrys staudtii, Uapaca paludosa, Xymalos baillon* and *Odyendyea gabonensis*. The above named sixteen plants are being investigated. Two plant families, Annonaceae and Apocynaceae were previously identified as the most common ingredients in the preparation of traditional malaria remedies in west and Central Africa. Isolated antiplasmodial compounds from members of these family were also found to be most active in our antimalarial screen. Extracts

from *Odyendyea gabonensis* a member of Rutaceae family were found to be very active at $IC_{50}$ of 92.9 and 95.1ng/ml for D6 and W2 respectively. Alkaloidal fractions from Rutaceae and Ancistrocladaceae also showed remarkable activity. . Follow-up studies have been carried out in some of these plants with confirmed antiplasmodial activity.

### 2.2 Opportunistic infection

There is no significant result from opportunistic infection tests during this report period.

### 2.3 Biological Screening Project

During the period August 1 1999 to July 2000, 104 plant extracts were screened in different screening assays in use in our laboratory at InterCEDD .The summary is as shown below:

A. NUMBER OF PLANT EXTRACTS SCREENED

| | |
|---|---|
| ANTI-MICROBIAL SCREENING | 44 |
| MINIMUM INHIBITORY CONCENTRATION (MIC) | 21 |
| BRINE SHRIMP LETHALITY ASSAY | 49 |

ANTI-MICROBIAL SCREENING

The anti microbial screening procedure involves first testing the plant extracts against the cultures of the test organisms at high concentrations of 2,000ug/ml and 5,000ug/ml . The plant extracts showing activity at 2,000ug/ml were further evaluated to determine their minimum Inhibitory concentration (MIC) and Minimum Bactericidal Concentration(MBC) values against the test organisms .The summary of the results for the 35 plant extracts are shown below :

Against

| | S.a | B .s | E.c | P.a | C.a |
|---|---|---|---|---|---|
| Number of plant extracts with anti-microbial activity | 19 | 15 | 4 | 6 | 1 |

8 plant extracts showed no activity.

Key

S.a (S.aureus ATCC 12600)
B.s (B.subtilis ATCC 6051)
P.a (P.aeruginosa ATCC 10145)
E.c (E.coli ATCC 11775)
C.a (C.albicans ATCC18804)

Extract Library
During the reporting period a total 340 extracts were processed for testing against other ICBG targets.

### 2.4 CNS Activity

The first batch of atypical anti-psychotics identified through ethnobotanical survey has been submitted to University of Miami, School of Medicine. The eleven extracts from eight plant species will be subjected to the CNS/ receptor transport screens. Active extracts will be classified according to their ligand binding profile. The preliminary results are expected.

### 2.5 Cystic Fibrosis

Yeast strains produced in Dr. Teem's laboratory at Florida State University were used in a bioassay to detect potential cystic fibrosis therapeutics in tropical plants in West and Central Africa. The yeast strains are designed to model the molecular defect responsible for cystic fibrosis, and are thus suitable for drug screening assays to identify compounds that reverse the defect. Personnel trained in the use of the assay from Dr. Teem's laboratory traveled to Nigeria and Cameroon with the materials needed to perform the bioassay. Plants from the tropical forest were screened using the assay, and those that contained active substances were identified and retested. Scientists within Nigeria and Cameroon were trained in the use of the bioassay. Candidate plants that were identified by the bioassay were used for the preparation of extracts at the BDCP laboratory at the University of Dschang in Cameroon. Active fractions were isolated in the BDCP laboratory, and sent to Dr. Teem's laboratory at Florida State University, where they were tested in secondary assays designed to measure the activity of small molecules on the function of the cystic fibrosis transmembrane conductance regulator chloride channel.
Result:
Plant Collection (Cameroon): All plants within a delimited area of tropical forest plants were tested. The ability to identify the plant species collected is imperative for the success of this strategy. In Cameroon, the collaboration with the ICBG AP-1 provided all the conditions for a successful plant collection, as we could test plant leaves collected at the Korup Forest Dynamics Plot (KFDP) and had the excellent assistance of two field botanists, Mambo Peter and Sainge Moses.

There are 481 species of woody plants in the 50 ha KFDP, the individuals with stem diameter larger than 1 cm are tagged and most are classified to the species level. This situation is ideal for random collection, since the identity of the plants tested can be readily obtained from the KFDP data set. Herbs and liana species were also screened and voucher specimens were collected and deposited in the herbarium in Mundemba (Tables 4-5).
Plant Collection (Nigeria): In Nigeria, plant collections were made from the Calabar region with the assistance of plant botanist, Mr. Ozioko. The total number of plants collected for screening was limited to only 200 due to limited time for collection.
Primary Screen Results (Cameroon):

Plant leaf collection was performed in the Korup National Forest and in the Ekondo-Kondo resettlement area. A total of 605 species of plants were screened, during a period of nine days, for the desired activity, 400 of those were tagged trees from the KFDP. The plants that yielded a positive result in either of the bioassays were subjected to a confirmatory test and are listed below.

Primary Screen Results (Nigeria):
In Nsukka, a well characterized plot of tropical forest with high biodiversity comparable to the Korup Forest Dynamics Plot (KFDP) in Cameroon is not readily available. Consequently, screening of fresh plant material was limited to various sites around Nsukka that were selected by Dr. Ozioko. On one occasion plants were tested from the Calabar region.

The primary resource for screening in Nsukka was the extract collection of InterCEDD. Scott Olenych screened the collection of extracts, using filter paper discs (soaked in samples of each extract) placed upon yeast indicator lawns. A total of 306 extracts from 130 plants were screened, during a period of two weeks. Extracts that were retested in a second round of testing and found positive indicated (+).

Bioassay-directed Fractionation and Secondary Screening (Cameroon)

Based on the best performance on the yeast bioassays, 11 species were chosen as a starting point to obtain extracts and eventually pure compounds. Plant species that yielded the best results in primary screen are: *Trichilia* sp., *Casearia* sp., *Bulbophylum* sp., *Homalium letestui*, *Sapium ellipticum*, *Hesteria parviflora*, MOKO (*Rubiaceae*), RUDO (*Rubiaceae*), *Drypetes molunduana*, *Ouratea vogelii*, *Garcinia ovalifolia*. Except for MOKO, *Sappium ellipticum* and *Bulbophylum* sp., bulk amounts of the other species have been collected by the BDCP in Mundemba.

Bioassay-directed Fractionation and Secondary Screening (Nigeria): The plants and plant extracts identified as positive in the yeast bioassays are shown below respectively.

Plants that tested positive by yeast bioassay are: *Crossopteryx febrifuga*, *Anthocleista djalonensis*, *Morinda lucida*, *Clausena anisata*, *Rubiaceae* sp., *Schwienickia americana*, *Canthium/Rubiaceae*, *Uvariastrum* sp.

Plant extracts that tested positive by yeast bioassay are: *Hyptis suaveolens*, *Schwenickia americana*, *Solenistemon monostachyas* and *Mormodica charanta*.

Plant extracts that tested positive by yeast bioassay are: *Hyptis suaveolens*, *Schwenickia americana*, *Solenistemon monostachyas*, *Mormodica charanta*, *Laggera pterodonta*, *Nauclea latifolia*, *Harungana madagascariensis*, *Gossypium arboreum*, *Garcinia kola*, *Detarium microcarpum*.

Unfractionated samples of extracts listed were brought to Florida State University where they are currently being tested for drug activity to correct the cystic fibrosis defect in mammalian cells. Bioassay directed fractionation of the extracts listed will be carried on by InterCEDD. Dr Lewis Ezeogu, Chibuike Eze, Emeka Ubadiniru, Richard Imah, Chioma Ezeobi, and Ngozi Asogwa (all members of InterCEDD) have all been trained in the use of the yeast bioassays, and will be involved in bioassay-directed fractionation studies. The extracts or fractions that yielded a positive result will be sent to Florida State University to be tested in mammalian cells.

**2.6 Phytomedicine Development**

In collaboration with the University of Jos, InterCEDD conducted standardization of medicinal plants used widely for the treatment of diseases. The Centre developed analytical standards, which will enable the regulatory agency (NAFDAC) to evaluate these herbal medicines for registration as phytomedicines. Pharmacognostical profile such as morphological description, extractive values, ash values, analytical standards for chemical composition, stability tests,

microbiological load, limits for the presence of heavy metals etc have been determined for the following herbs: *Aframomum melegueta, Picralima nitida, Morinda lucida, Enantia chlorantha, Uvaria chamae, Gongronema latifolium, Cola accuminata, Combretum micranthum, Garcinia kola, Zingiber officinale Ocimum gratissimum.*

Sample copies of certificate of analysis issued by InterCEDD for some of the commercial samples examined by the Centre are reproduced in Fig 2-6 (See attachment).

**2.7 Antiviral**

A total of 35 samples of higher plants have been submitted for testing for their antiviral activity at the Southern Research Institute (SRI). The thirty-five plants extracts were chosen from 29 species basis on an ethnomedical survey. The extracts were tested against five viruses such as poliovirus, hepatitis and herpes The results of the antiviral testing is attached. Follow-up fractionation would be carried out on those plant extracts that showed antiviral activity. Previously we have screened 25 extracts for anti-HIV activity. Two plant extracts showed potential activity in this test. Bioassay-directed fractionation of the most active extract led to the isolation and characterization of two active compounds The same constituent showed *in vitro* activity against *ebola* virus.

**2.8 Antileishmanial activity**

Evaluation of the antileishmanial activity of 130 extracts representing 52 plant species implicated in traditional medicine enabled the identification of new antileishmanial chemotypes that proved highly active and are radically different from the existing drugs. We have continued with dereplication of the antileishmanial compounds from the two plants in our recent antiprotozoal patent application. Sakuranetin which is the most active antileishmanial compound from *Eupatorium odorantum* and labdane diterpene from *Aframomum daniellii* have been isolated in large quantities to enable *in vivo* bioassay.

**2.9 Antitrypanosomal and Antitrichomonal Activity**

During this period, 21 extracts were screened against one *Trypanosoma brucei* strain and three *Trypanosoma rhodesiense* strains. Eight of these had $IC_{50}$ values of $\leq 0.1$ to $< 20$ µg/ml, and were of interest for further studies. Four extracts from previous studies were tested *in vivo* in a *T. brucei* mouse model, at up to 50 mg/kg/day i.p. x 3 days. None prolonged the life-span of infected animals. Additionally, based on trypanosome sterol requirements and evidence of plant sterols in active plant extracts, five anti-hypercholesteremic agents used in clinical medicine were tested in the trypanosome mouse model. None prolonged the life-span of infected animals at up to 100 mg/kg/day for 3 days.

16 extracts were tested vs. metronidazole-sensitive and -resistant isolates of *Trichomonas vaginalis* and a *Tritrichomonas foetus* isolate. Of these, five had MIC values of $\leq 0.1$ mg/ml including an extract of *Dracaena mannii*, which gave MIC values of 0.0125 to 0.006 mg/ml. These studies are continuing, with increased emphasis on *in vivo* testing of more highly purified plant extracts in an effort to determine the active agent(s) in these extracts.

a) Screening of plant extracts for *in vitro* growth inhibitors vs. African trypanosomes. A total of 25 primary and secondary plant extracts were received from InterCEDD. Of these sufficient quantities of 21 allowed testing against all four trypanosome isolates in a standard screen (Table 6). The data indicated that eight agents had significantly low $IC_{50}$

values ($\leq 0.1$ to $\leq 20$ µg/ml) as to be of interest for continued study. The origins of these extracts were *Cryptolepis sanguinolenta, Platex vellous, Fagara lemairei, Erythrina senegalensis, Glossocalyx brevipes, and Dorsternia barteri*.

An additional 14 extracts were supplied by BDCP lab.at the University of Dschang (Table 7). All of these had $IC_{50}$ values of $\leq 20$µg/ml, and included methanol and water extracts of *Aframomum aulocacarpus* and *Glossocalyx brevipes*, as well as extracts of other local plants. Data for compounds Al+6, Al+7, ND1, ND2, and ND5 is incomplete, since the *T. b. rhodesiense* screens have not yet been run.

b) *In vivo* studies using mouse model infections. A total of 17 extracts were judged sufficiently active for screening *in vivo* (Table 8). Table 8b outlines the *in vivo* screening studies with plant extracts. Four extracts giving low $IC_{50}$ values in a previous study were run using the *T. b. brucei* Lab 110 isolate in a standard screen. Agents were suspended in 2% methylcellulose + 0.5% Tween 80, and administered i.p. for 3 days. SU-1461 was also given p.o. for 3 days. None of the extracts protected the mice and they died at the same time as infected, untreated controls. The remaining *in vivo* screens will be set up once additional supplies of extracts are received from InterCEDD.

c) Sterol synthesis inhibitors: link to hemoflagellate parasites. Some phytosterols act as cholesterol analogs and can disrupt sterol uptake and synthesis in hemoflagellate parasites leading to death of the parasite. African trypanosomes depend on exogenous cholesterol. Coppens et al. showed the inhibitor Synvinolin (symvastatin) potentiates growth inhibition *in vitro* of *T. brucei* in the presence of drugs interfering with exogenous supply of cholesterol and that inhibition could be reversed by squalene, mevalonate, or cholesterol. Coppens and Courtoy showed that *T.brucei* procyclic forms (insect vector) contain egosterol synthesized *de novo*, and also incorporate exogenous cholesterol into membranes. Culture-adapted parasites also grow more rapidly in the presence of LDL.

d) *In vitro* activity of plant extracts vs. *Trichomonas* spp. A total of 16 plant extracts were screened against *T. vaginalis*, metronidazole-sensitive (C1-NIH) and resistant (CDC-085) isolates, while 11 extracts were tested vs. *Trichomonas foetus* (KV1). All of these agents gave $IC_{50}$ values $< 1$ mg/ml and several (SU-1460, 1461, 1464) were active at $< 0.1$ mg/ml (Table 9). One very interesting finding was the very low $IC_{50}$ obtained for SU-1460 vs. the metronidazole-resistant strain CDC-085. This value, 0.0015 mg/ml was 100-fold less than that found for the standard sensitive strain (C1-NIH). Of significance was the low $IC_{50}$ values obtained for the *T. foetus* extracts (SU-1461, 1464). Further studies will examine secondary extracts of the active extracts in an attempt to improve activity and specificity. Additional studies will also involve *in vivo* testing through use of a sub-dermal trichomonas model infection in laboratory mice.

**2.8 Cytotoxicity and anti-cancer screens:**

In the current year of activity 78 plant extracts from Nigeria and Cameroon were screened for cytotoxicity in human cancer cell lines. These extracts were from plants used traditionally for antiparasitic activity, and a surprisingly high 85% were appreciably cytotoxic toward the human cancer cells. Active extracts were screened further for evidence of anticancer utility. Of the extracts tested 19 or 23% exhibited activity indicative of DNA strand break activity. Experience has shown that the differential cytotoxicity profiles suggest specific mechanisms of DNA

damage. Differentials in xrs-6 cells are indicative of direct chemical double strand scission, or topoisomerase II poisoning. Differentials in EM9 cells are indicative of single strand scission (usually via reactive oxygen species), or topoisomerase I poisoning. None of the extracts tested exhibited enhanced activity indicative of covalent DNA modification. Extracts exhibiting these activities (e.g. 848 – see spreadsheet in appendix) are currently being tested in purified DNA and human topoisomerase assays *in vitro*.

Several extracts were identified with specified effects on the cell cycle. The most striking effect is that of extract SU-799, which causes aneuploidy in CHO cells.

The key research accomplishments are:

- 78 extracts were screened for cytotoxicity
- 67 (85%) of the extracts were cytotoxic at 100ug/ml or less.
- 19 (23%) of the extracts appear to damage DNA or interfere with DNA metabolism
- 11 of 14 extracts appear to damage DNA or interfere with DNA metabolism
- The majority of these data were presented at the ICBG annual meeting in Douala, Cameroon, and February 2000.
- This research has supplied rotation projects and contributed to the training of two graduate (Ph.D.) students at the University of Utah.

### 2.9 Antituberculosis

Eighteen extracts from InterCEDD inventory of plants used in the treatment of parasitic diseases were submitted for antituberculosis evaluation at the Southern Research Institute. Primary (level 1) and secondary (level 2) results showed that four of the extracts were very active as shown in Table 10). The active extracts include *Eugenia uniflora, Enantia chlorantha, Dorstenia multiradiata* and *Chasmanthera dependens* . Bioassay-directed fractionation of the four extract will be continued during the next project period.

## SUMMARY OF ICBG DRUG DEVELOPMENT LEADS

| DISEASES | SAMPLES TESTED | LAB. | ACTIVITY % | LEADS |
|---|---|---|---|---|
| Malaria | 720 | WRAIR | 397(55%) | 27 |
| Leishmaniasis | 130 | WRAIR | 52(39%) | 6 |
| Cytotoxicity | 98 | UTAH | 83(85%)* | 11 |
| Viral | 30 | SRI | 16(63%) | 2 |
| Opportunistic infection | | | | |
| Cryptosporidium | 22 | NIAID | 7(31%) | 2 |
| Toxoplasmosis | 22 | NIAID | 6(27%) | 2 |

| | | | | |
|---|---|---|---|---|
| Trypanosomiasis | 61 | PACE | 47(77%) | 15** |
| Trichomonas | 41 | PACE | 26(63%) | 10 |
| Tuberculosis | 18 | TAAF | 4(22%) | 3 |
| Cystic Fibrosis | 805 | Florida State U. | 65 (8%) | 3 |

* active at 50ug/ml
** potential anticancer plants
one of the plant isolate showed *in vitro* activity against ebola virus

## 3 SUSTAINABLE DEVELOPMENT:

During this project reporting period, the objectives was to continue the ethnobotanical studies and socio-economic evaluation of plant species in areas not yet covered. With the support of CARPE, we also evaluated the use of phytomedicines as an economic incentive for biodiversity conservation in Cameroon.

### 1. Ethnobotanical Studies

Ethnobotanical studies were conducted in Imo State and Ukwa-Ndoki areas of Rivers State of Nigeria. In Cameroon, the project teams collected data among the Bafut, Sabga and Oku tribes. With the entries from this work, we have now completed the first phase of our documentation of the Ethnobotany of Cameroon. The database on Cameroonian medicinal plants showed that 434 plant species are most commonly used in the preparation of traditional medicines. The data, which has been entered as part of the AFRICMED Database, includes summaries of all the available ethnobotanical information on Cameroonian ethnobotany. It contains information on the botanical classification, method of use and the local names for the plants in at least 6 vernacular languages. The species entries in the AFRICMED database increased by 214 during the project period.

### 2. Socio-Economic Evaluation of Non-Timber Forest Products

*Exploitation and Use of Non-Timber Forest Products:*
Information collected shows a wide degree of access to and use of community forests in food consumption and its importance in the diet, plant medicines and for house building, household and agricultural equipment, fire wood, fodder and in trade and processing activities. Consumer surveys were undertaken to assess the demand for bamboo, cane and raffia palm products and the extent to which people rely on herbal medicines.

a. *Medicinal Uses:*
Data from the BDCP small plots is dominated by forest medicines. These are highly valued as sources of natural medicines, which are essential components of health treatments throughout the region. They are the main medicines used by a vast majority of all classes of people and despite the many different healing practices - they are still commonly used in conjunction with mystical and ritual practices. The sacred value attached to some plants like *Adenia lobatal, Warrior liana* and royal reserves confirms the link between healing and spiritual values. Survey results from local communities testify that about 70% of the sample population rely on wild medicinal plants as main source of treatments. The general belief is that certain diseases can best be treated by

traditional plant cures notably epilepsy, mental disorders and spiritual problems. It was noticed that the use of herbal medicines is not restricted to specialists. By far the most common users of medicinal plants as self-administered first aid are the women who play a central role in the first aid treatments of their children. This indigenous knowledge is passed on in families and even young ones are well versed in the use of medicinal plants as first aid. Most herbal medicines encountered were given both as curative and preventive. The latter were often added to soup and used as blood tonics or to increase the lactation of a newly delivered mother. Others are taken as infusions, decoction or enemas. It was definite that the medicinal uses of forest plants is widely known as some trees were left on farms and back gardens because of their medicinal properties.

*b. Non-Medicinal Uses:*

From the 547 plants collected through household questionnaires it was certain that the forest contributes to all aspects of rural life providing bush foods fodder fuel, building material, household items as well as many intangible benefits such as the cultural and symbolic (figurines and decorative artifacts). Each community is unique in its type of building materials due to the differences in the availability of the plants in their environment. Although forests maintain environmental stability as well as sources of income, they furnish bush food for animals and humans. This bush food is consumed occasionally by women and children who use leaves for food wrapping, wild spices, as well as scientist and hunters searching for game and medicinal plants. Many bush foods are either flavorants (*Xylopia* sp.; *Xymalos monospora*; *Piper guineense*; *Piper capense; Aframomum melengueta; Aframomum_prunuism*; and *Afrostyrax lepidophyllus* or wild fruits of *Aframomum danielli*; *Annona gsenegalensis* etc. Mushroom gathering was witnessed as a serious hobby and only three species were collected - *Termitomyces striatus Agaricus* sp. And *Polypomus* sp. Collectively these flavorants add diversity and flavor to the diet as well as provide proteins, energy, vitamins and minerals. They are of particular importance to patients suffering from blood wasting diseases and lactating mothers who compared to adults consume greater quantities of food, to acquire additional vitamins when they are most vulnerable to anemia.

There is widespread use of items made from NTFPs in daily life, but the range of the goods varies within and between the tribes. The highly valued household item in most villages is the pestle made from *Raphia vinifera* and *Harungana madagascariensis*. Survey results from BDCP projects shows that it was ranked as the most important item above all other forest products. Formerly pestles used to be made also from *Pygeum africamum* but with the discovery of the use of the plants for the treatment of prostrate cancer, over harvesting of its bark have limited its use. Other essential items in the kitchen include spoons, grinders, baskets, mats and chairs. Forest resources are also widely used for making musical instruments, carved tools, building of bridges, pit latrines, canoes, sleeping and fencing mats, as well as utensils, sponges, brooms, and sandpaper. They also supply material for agricultural equipment such as hoe, axe and cutlass handles, yam stakes etc. One of the most exhaustible plants observed was the cane *Ariendinaria alpina* that is almost extinct. The trade in this climbing plant is enormous and there are no campaigns for its replacement.

Leaves from several species are widely used by traders and food sellers for packing material. The leaves most commonly used are from the Musaceae and Marantaceae families. The former is collected from around houses and the other from swampy sites. The former must be warmed over a flame to prevent from tear. Marantaceae leaves are strong, durable, impermeable and able to withstand heat, all of which makes them invaluable to food sellers and traders. These leaves are collected regularly by women for the wrapping of fish, salt, meat, and cooked food

notably pounded cocoyams. They also impart flavor to the cooked food as well as its preserving qualities.

*c. The Role of Women:*

The involvement of women is limited as some traditions restrict them from touching certain plants like *Ceiba petandra* (Silk cotton), *Dracaena deisteliana* (Peace plant) or from uprooting cultural plants - *Raphia vinifera* (Raffia palm), *Elaeis guineensis* (oil palm) and *Cola acuminata* (Kola nuts). This is especially the case during the menstruation cycle. In spite of this, the gender distribution of roles shows that women play an important role as food providers for their families. They are responsible for firewood and first aid treatments for their children, as they are the first to diagnose and treat their children. Evidence from BDCP projects shows an intimate relationship with the forest. This is reflected in their unique vocabulary and diversified knowledge of all sorts of plants. This is further enhanced through wood fetching, farming, and primary health care experiences of their children's health. In large part, they can be said to be the custodians of scarce and lost crops of the Cameroons. Their knowledge of herbs for fertility disease is unique. Apart from these restrictions most Bafut and Oku women's income come from the sales of wild spices food wrappers, food crops and vegetables.

*Marketing of NTFPs:*

Through a review of existing data, the following medicinal plants have been identified in the market for non-timber forest products:

| **Name of Plant** | **Plant Parts Traded** |
|---|---|
| *Adansoia digitata* | Fruits, seeds, leaves |
| *Aframomum melegueta* | Seeds |
| *Agave sisalana* | Leaves, Stems |
| *Ageratum conyzoides* | Whole plant |
| *Allanblackia* | Seeds |
| *Aloe Vera* | Leaves |
| *Alstonia congensis* | Barks, latex |
| *Anacardium occidentale* | Barks, fruits, seeds |
| *Ananas comosus* | Fruits, stems, leaves |
| *Annona muricata* | Seeds, leaves |
| *Arbrus precatorious* | Leaves, seeds, roots |
| *Argemone mexicana* | Leaves, full plant |
| *Ariza sativa* | Seeds |
| *Artemisia afra* | Root |
| *Artocapus communis* | Fruits, seed, bark |
| *Arocarpus herterophyllus* | Fruits, seeds |
| *Azadirachta indica* | Leaves |
| *Bixa orellana* | Seeds |
| *Butyrospermum parkii* | Seeds |
| *Cajanus cajan* | Seeds |
| *Calophylum inophyllum* | Seeds |
| *Camelia sinensis* | Leaves |
| *Cananga odorata* | Flowers, fruits |
| *Canavalia ensiformis* | Seeds, fruits |

| | |
|---|---|
| *Capsicum frutexens* | Fruits |
| *Carapa procera* | Seeds, barks |
| *Carica papaya* | Fruits, leaves |
| *Cassia alata* | Leaves |
| *Cassia occidentalis* | Leaves |
| *Catharanthus roseus* | Roots, leaves |
| *Centella asiatica* | Full plant |
| *Chenopodium ambrosioides* | Leaves, flowers |
| *Chrysantellum americanum* | Full plant |
| *Cocus nucifera* | Fruits |
| *Cola nitida* | Seeds |
| *Combretum micranthum* | Leaves |
| *Costus afer* | Leaves, stems |
| *Crotalaria sp.* | Root |
| *Croton sylvaticus* | Bark |
| *Croton zabesicus* | Leaves |
| *Cucurbita maxima* | Seeds |
| *Cymbropogon citrates* | Leaves |
| *Datura mettel* | Leaves |

There is sufficient data to illustrate the fact that forests as a whole are extremely important to local communities in their role as environmental and economic buffers. They provide subsistence money-hence a need for setting-up a business in NTFP market. From this preliminary assessment, there is need for further investigation on the impact of the changing nature of the forest environment over time, on local NTFP trade, and sustainability of a phytomedicine industry in the region.

3. Phytomedicine Development

Surveys of local markets are still on-going to assess the extent of trade in selected focal species. New medicinal plants not encountered in the first survey were observed in the present studies. For example, *Pachyelasma* sp –a rare medicinal plant used only by professional healers was found in most ethno-pharmaceutical stalls but was not listed in the six previous ethnobotanical surveys. Some of the families that are more frequently used include:
*Apocynaceae, Annonaceae, Asteraceae ,Euphorbiaceae , Melicaceae , Zingiberaceae, Piperaceae, Fabaceae, Bignoniaceae , Liliaceae/Amaryllidaceae ,Rutaceae Caesalpinioideae, Pittosporaceae, Ericaceae, Guttiferae, Mimosaceae, Burseraceae*

It was found from the survey that there was no uniformity in the frequency of the use of medicinal plants among different ethnic groups. Each tribe has its own unique medicinal focal species based on the use of local flora for the treatment of diseases that are prevalent in that area. Sometimes a village may possess very high degree of expertise in the treatment of a particular disease or a group of diseases and healers from that village is consulted from neighboring villages for knowledge of medicinal plants for that disease(s). Most of the focal species are herbaceous plants which are usually heavily exploited but are neither sold in local nor international markets. Traditional healers seldom buy their herbs from commercial herb

vendors, which minimizes any competition between local use and the possible demand from phytomedical industry.

The reasons given by the healers for not purchasing their herbs from local commercial markets include:

1. In the course of their being harvested, herb traders violate some ancestral norms pertaining to medicinal plants.
2. The origin of their profession was never negotiated on the basis of money; consequently, the rules of their profession do not warrant any monetary involvement in the use of medicinal plants for treatment.

Common spices and herbs, however, are obtained from herb sellers. These: *Aframomum* sp, *Capsicum* sp, *Piper* sp, castor oil, palm kernel oil and perennial organs such as *Crinum* sp., *Gladiolus* sp. and assorted mixtures of potpourri used for cleansing their consultation offices/alters.

**KEY RESEARCH ACCOMPLISHMENT:**

1. Extracted and tested 120 plants for malaria, 98 samples for cytotoxocity, 30 samples for HIV, 41 samples for trichomonas, 18 samples for Tuberculosis and 805 for Cystic Fibrosis.
2. Screened 104 plant extracts against anti-microbial screens and brine shrimp lethality assay.
3. Processed and added 340 extracts to the Extract bank for testing against ICBG disease target.
4. Standardized medicinal plants used widely for the treatment of diseases.
5. Developed analytical standard in collaboration with Nigerian regulatory agency, National Agency for Food and Drug Administration and Control (NAFDAC), to evaluate these herbal medicines for registration as phytomedicines.
6. Pharmacognostical profile such as morphological description, extractive values, ash values, analytical standards for chemical composition, stability tests, microbiological load, the presence of heavy metals have been determined for the 11 herbs.
7. Ethnobotanical studies were conducted in Imo and Rivers states of Nigeria.
8. Completed the first phase of our documentation of the ethnobotany of Cameroon.
9. Continued socio-economic study of non-timber forest products.
10. Data on 547 plants were collected through household questionnaires.

## 7. REPORTABLE OUTCOMES

**Peer Reviewed Articles**

B.G. Schuster, J.E. Jackson, C.N. Obijiofor, C.O. Okunji, W. Milhous, E. Losos, J.F. Ayafor and M.M.Iwu (1999), Drug Development and Conservation of Biodiversity in west and Central Africa: A Model for Collaboration with Indigenous people. *Pharmaceutical Biology* (37) 1-16.

Tchuendem MH, Mbah JA, Tsopmo A, Ayafor JF, Sterner O, Okunji CO, Iwu MM, Schuster BM (1999), Anti-plasmodial sesquiterpenoids from the African Reneilmia cincinnata;. Phytochemistry Nov;52(6):1095-9

Maurice M. Iwu, Angela R. Duncan, Chris O. Okunji (1999), New Antimicrobials of Plant origin New Crops and New Uses, Prospectives in New Crops and New uses pp. 447-452.

**Presentations at Meetings, Conferences**

Iwu M. Maurice, Biodiversity Utilization and Conservation in West and Central Africa; being a plenary lecture delivered at the 2nd IUPAC International Conference on Biodiversity, 11-15th July 1999, Belo Horizonte, MG, Brazil

Iwu M. Maurice, Okunji O. Chris, Ayafor F. Johnson, Akubue, P.I, Jackson E. Joan, Tally D. John, Cyrus Bacchi and Schuster B.G:; Antiprotozoal Agents From African Medicinal Plants Based on Ethnomedical Leads; being an invited paper delivered at the 2nd IUPAC International Conference on Biodiversity, 11-15th July 1999, Belo Horizonte, MG, Brazil

Okunji O Chris. Skanchy J. David, and Iwu M. Maurice, Quantitative Estimation of Kolaviron In *Garcinia kola* Preparations Using Capillary Electrophoresis, paper delivered at the American Society of Pharmacognosy Interim Meeting , April 29-May 1, 1999 Grand Casino convention Center and veranda Hotel Tunica, Mississippi

Akubue, P.I., Onyechere, C., Obijiofor, C., Duncan, A.R., Okunji, C.O., Azuine, M.A., Iwu, M.M., Clinical Evaluation of Kola in Human Volunteers, a paper delivered at the American Society of Pharmacognosy Interim Meeting , April 29-May 1, 1999 Grand Casino convention Center and veranda Hotel Tunica, Mississippi

Okunji O. Chris, Jackson E. Joan, Tally D. John and Iwu M. Maurice; *In vitro* Antileishmanial activity of Labda-8(17),12-diene-15,16-dial from *Aframomum daniellii* K.Schum. (Family Zingiberaceae), Internati being a poster presentation at the International Conference on Ethnomedicine and Drug Discovery, Holiday Inn, Silver Spring, Maryland, USA November 3-5, 1999.

Okunji O. Chris, Skanchy J. David and Iwu M. Maurice, Challenges and Issues involved in the Standardization of *Garcinia kola* Formulations; being a plenary paper delivered at the International Conference on Ethnomedicine and Drug Discovery, Holiday Inn, Silver Spring, Maryland, USA November 3-5, 1999.

Simon Efange, Natural products- a continuous source of inspiration for the medicinal chemist; being a plenary paper delivered at the International Conference on Ethnomedicine and Drug Discovery, Holiday Inn, Silver Spring, Maryland, USA November 3-5, 1999.

Chioma Obijiofor, Integrating African Ethnomedicine into Primary Healthcare in South-Eastern Nigeria: Recommendations for the Government of South-Eastern States; being a poster delivered at the International Conference on Ethnomedicine and Drug Discovery, Holiday Inn, Silver Spring, Maryland, USA November 3-5, 1999.

Schuster B.G, Obijiofor C.N and Iwu, MM, International Cooperative Biodiversity Group on Drug Development and Conservation of Biodiversity in West and Central Africa; being a

plenary paper delivered at the International Conference on Ethnomedicine and Drug Discovery, Holiday Inn, Silver Spring, Maryland, USA November 3-5, 1999.

Anthony Onugu, Sustainable Development and Poverty Alleviation in Africa, presented at Global Biodiversity Forum, organized by CBD Secretariat, Nairobi, May2000.

Appolinaire Tsopmo, Flavonoids and Urea Derivatives of some Cameroonian Spices: *Afromomum hanburyii, Dorstenia Poinsettifolia, D. manii* and *Pentadiplandra brazzana*, a Doctorate thesis presented at the Department of Chemistry, University of Dschang, Cameroon

Tchuendem Kenmogne Maguerite-Hortence, Terpernoids and Lignans from some Cameroonian spice, a Doctorate thesis presented at the Department of Organic Chemistry, University of Dschang, Cameroon.

**Patents:**

U.S. Patent Application Serial No. 09/382,128
Filing Date: August 24, 1999
For: Antifungal and Antiparasitic Compounds
By: J.E. Jackson, M.M. Iwu, C.O. Okunji, C. Bacchi, J.D. Tally, Jr., J.F. Ayafor.

U.S. Patent Application Serial No. 09/428,203
Filing Date: October 27, 1999
For: Plant-derived Antiparasitic Antifungal Compounds and Methods of Extracting the Compound.
By: C.O. Okunji, J.E. Jackson, M.M. Iwu, C. Bacchi, J.D. Tally, Jr., J.F. Ayafor.

## CONCLUSIONS:

BDCP successfully accomplished the scope of work outlined for this project reporting period. During the next project period work will continue on extracting plants used in ethnomedicine and large scale extraction of the following species for the therapeutic categories indicated:

**Leishmania**
*Eupatorium odoratum.*
*Aframomum danellii*

**Anticancer**
Uvaria chamae
*Araliopsis tabouensis*
*Aframomum melegueta*
*Diodea scandens*
*Dorsternia barteri*

**Malaria**
*Picralima nitida*
*Araliopsis tabouensis*
*Morinda lucida*
*Enantia chlorantha*
*Spathodea campanulata*
*Synclisia scarbrida*
*Uvaria chamae*
*Cryptolepis sanguinolenta*
*Glossocalyx brevipes*
*Cleistopholis patens*
*Leidobotrys staudtii*
*Pachypodanthium staudtii*
Odyendyea gabonensis
*Uapaca paludosa*
*Xymalos baillon*
*Ancistrocladus barteri*

**Trypanosomiasis**
*Glossocalyx brevipes*
*Fagara lemairei*
*Picralima nitida*

**Antituberculosis**
*Chasmanthera dependens*
*Dorstenia multiradiata*
*Enantia chlorantha*

**Trichomonas**
*Gongronema latifolium*
*Albizia ferruginea*

In collaboration with AP-1, random collection will be initiated from both the large plot in Cameroon and the small biodiversity plots.
In the area of Sustainable Development, the following work plan will be used:
a. Ethnobotanical studies: With the completion of ethnobotanical studies in Cameroon, during the next project period greater emphasis will be in the analysis of the data collected and the publication of the report. Electronic versions of the report will be available for collaborating institutions both in Africa and the United States. The Nigerian ethnobotanical studies which cover a more heterogeneous population than Cameroon will be continued with greater emphasis on the Niger Delta region of the study site.
b. Plant Collection: In collaboration with AP-1, random collection will be initiated from both the large plot in Cameroon and the smaller 1 ha plots in Nigeria and Cameroon. Random collection success rates will be compared to the ethnomedical collections for a number of screens. Target species for drug development will be collected in large quantities to enable AP-2 to isolate sufficient quantities of compound for *in vivo* studies.

c. Socio-Economic Valuation Studies: Although the importance of the forest was prominent through its focus on NTFP, these resources hardly feature in forest management planning and yet NTFP provide the main link between the communities living by the forest and the Forestry Department. Since NTFPs cannot be boxed under one management regime, management systems that sustain and develop the value of the forest for local communities, is a primary basis for sustained local interest in forest long-term management. Whereas natural resource management objectives are often limited to forests, what happens outside forest resources, equally influences what happens inside it. Therefore environmental changes, which affect land-use practices outside the forest, may have a direct impact on how people use it. The following questions then arise:

- What are the focal species in NTFP trade, and on what criteria should this determination be based?
- What are the forward and backward mechanisms for integrating this discordant activity into a viable industry?
- What are the implications of this for access to natural resources and macroeconomic planning?
- What mechanisms exist for linking this activity to local conservation and development effort?
- What is the internal scientific capacity in the region to support this industry?

These are the questions that will be addressed in the next phase of this project.

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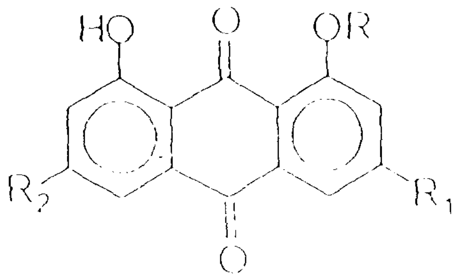
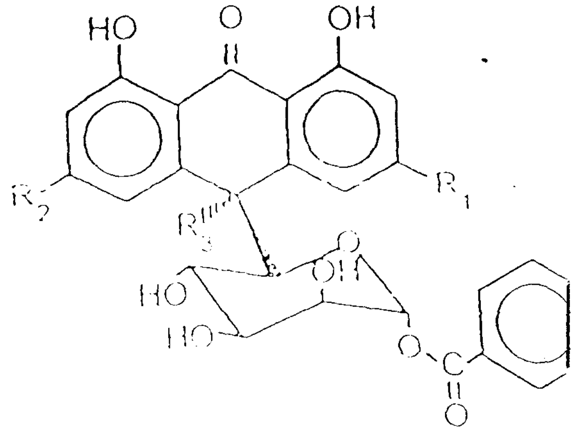
# APPENDICES

**Figure 1**



| | | R | $R_1$ | $R_2$ |
|---|---|---|---|---|
| (1) | Chrysophanol | H | $CH_3$ | H |
| (ii) | Emodin | H | OH | $CH_3$ |
| (iii) | Aloe-emodin | H | $CH_2OH$ | H |



| | | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|
| (iv) | Uveoside (1) | $CH_3$ | H | H |
| (v) | 10-epi-uveoside (2) | H | $CH_3$ | H |
| (vi) | Desoxisaroside (3) | $CH_3$ | OH | H |
| (vii) | Desoximayoside (4) | OH | $CH_3$ | H |
| (viii) | Mayoside | OH | $CH_3$ | OH |
| (ix) | Saroside | $CH_3$ | OH | OH |

**Table 1** *In vitro* Antimalarial Activity of 9 Anthraquinones Against Clones of *Plasmodium falciparum*

| | Compounds | Lab No. | IC50 (ng/ml) | |
|---|---|---|---|---|
| | | | $D_6$ clone | $W_2$ clone |
| (i) | Chrysophanol | SU-1858 | >5000 | >5000 |
| (ii) | Emodin | SU-1860 | 11167.34 | 5584 |
| (iii) | Aloe-emodin | SU-1859 | 7364.55 | 8434.52 |
| (iv) | Uveoside | SU-1856 | 1177.4 | 1248.55 |
| (v) | 10-epi-Uveoside | SU-1855 | 2532.46 | 2240 |
| (vi) | Desoxisaroside | SU-1854 | 5660.86 | 4757.5 |
| (vii) | Desoximayoside | SU-1867 | 5835.5 | 4803.5 |
| (viii) | Mayoside | SU-1861 | >5000 | 27000 |
| (ix) | Saroside | SU-1862 | 9487.09 | 9763.4 |

TABLE 2

**Bioassay-Guided Chromatographic Fractionation of Two Antimalarial Plants**

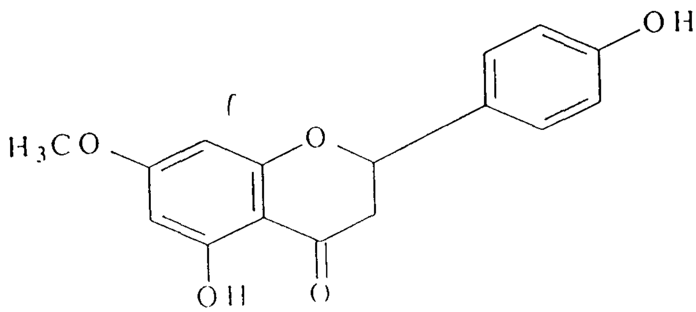
| **Plant source** | **Bott No.** | **Lab. Code** | **$IC_{50}$ (ng/ml) $D_6$ clone** | **$W_2$ clone** |
|---|---|---|---|---|
| Cleistopholis patens | BP10611 | CPA-I-3A | 6833.548 | 4191.173 |
| | BP10620 | CPA-I-3P | 4994.074 | 3216.473 |
| | BP10639 | CPA-I-3C | 2348.351 | 2044.26 |
| | BP10648 | CPA-I-3D | 5949.981 | 4354.625 |
| | BP10657 | CPA-I-3E | 2784.202 | 2631.749 |
| | BP10666 | CPA-I-3F | 2516.673 | 2511.741 |
| | BP10675 | CPA-I-3G | 0.0000 | 0.0000 |
| | BP10684 | CPA-I-3H | 0.0000 | 0.0000 |
| | BP10693 | CPA-I-3I | 26045.64 | 0.0000 |
| | BP10700 | CPA-I-3J | 17148.91 | 0.0000 |
| *Pachypodanthium staudtii* | BP10719 | PSA-I-15A | 1605.753 | 676.813 |
| | BP10737 | PSA-I-15C | 252.201 | 165.565 |
| | BP10746 | PSA-I-15D | 2175.274 | 1658.75 |
| | BP10755 | PSA-I-15E | **680.534** | **669.888** |
| | BP10764 | PSA-I-15F | 3918.673 | 3286.724 |
| | BP10773 | PSA-I-16A | 6909.135 | 7235.785 |
| | BP10782 | PSA-I-16B | 504.104 | 719.633 |
| | BP10791 | PSA-I-16C | **571.496** | **414.145** |
| | BP10817 | PSA-I-16D | **291.785** | **273.126** |
| | BP10826 | PSA-I-16E | **117.603** | **119.773** |
| | BP10835 | PSA-I-16F | 1065.167 | 809.018 |
| | BP10844 | PSA-I-13A | 1423.126 | 971.072 |
| | BP10853 | PSA-I-13B | **545.961** | **524.731** |
| | BP10862 | PSA-I-13C | 236.598 | 119.279 |
| | BP10871 | PSA-I-13D | **118.239** | **119.312** |
| | BP10880 | PSA-I-13E | **124.676** | **124.146** |
| | BP10899 | PSA-I-13F | 787.433 | 600.485 |
| | BP10906 | PSA-I-13G | 3269.381 | 2171.51 |
| | BP10915 | PSA-I-13H | **662.038** | **782.892** |
| | BP10924 | PSA-I-13I | 1925.808 | 1682.23 |
| | BP10933 | PSA-I-13J | 12554.71 | 9245.53 |
| | BP10942 | PSA-I-13Q | 0.0000 | 0.0000 |
| | BP10951 | PSA-I-17A | **171.889** | **175.638** |
| | BP10960 | PSA-I-17B | **351.242** | **333.389** |
| | BP10979 | PSA-I-17C | **261.702** | **257.344** |
| | BP10988 | PSA-I-17D | **173.101** | **101.377** |
| | BP10997 | PSA-I-17E | **111.394** | **83.083** |

TABLE 2 continued

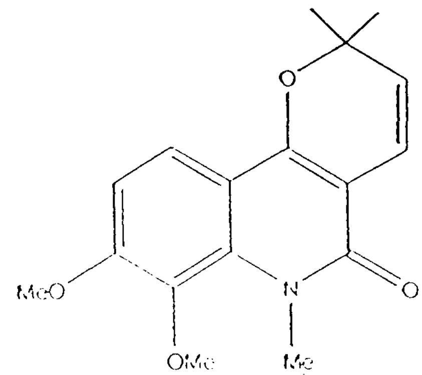
**Bioassay-Guided Chromatographic Fractionation Results Contd**

| Plant source | Bott No. | Lab. Code | $IC_{50}$ (ng/ml) $D_6$ clone | $W_2$ clone |
|---|---|---|---|---|
| | BP11001 | PSA-I-17F | 1393.907 | **922.221** |
| *Pachypodanthium staudtii* | BP11010 | PSA-I-18A | 15893.02 | 14850.5 |
| | BP11029 | PSA-I-18B | 8213.163 | 7872.953 |
| | BP11038 | PSA-I-18C | 2185.643 | 3665.943 |
| | BP11047 | PSA-I-18D | 2011.69 | 4967.47 |
| | BP11056 | PSA-I-18E | 0.0000 | **128.155** |
| | BP11065 | PSA-I-18F | 0.0000 | **934.186** |
| *Cleistopholis patens* | BP11074 | CPA-I-2A | 3135.264 | 9282.556 |
| | BP11083 | CPA-I-2C | 0.0000 | 0.0000 |
| | BP11092 | CPA-I-2D | 4138.018 | 14783.270 |

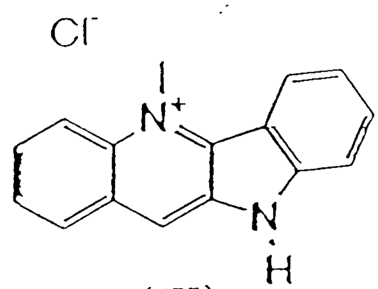
**Table 3**



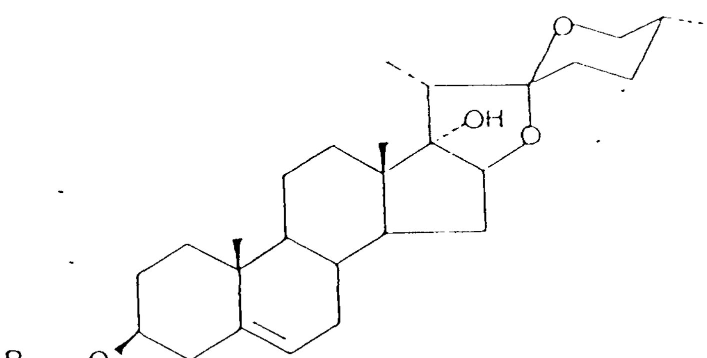
(X) Sakuranetin



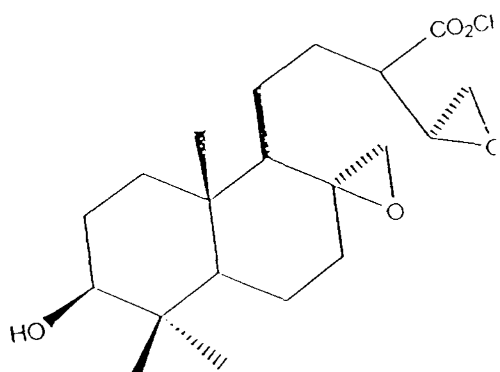
(XI) AT 7
Vepresine



(XII)
Crytolepine



Mannispirostan A



(XIX) AZ 2
Afromodal

(XIII) R = 3β-O-[{α-L-rhamnopyranosyl (1→2), α-L-rhamnopyranosyl (1→3)}-β-D-acetylglucopyranosyl]-17α-hydroxy-spirost-5-ene

STRUCTURES OF ISOLATED ANTIPROPTOZOAL COMPOUNDS.

**Table 4 Trees screened at Korup National Park**

| Family | No species | No Positives | Family | No species | No Positives |
|---|---|---|---|---|---|
| Acanthaceae | 2 | 1 | Moraceae | 5 | 1 |
| Anacardiaceae | 14 | 1 | Myristicaceae | 5 | |
| Anisophylaceae | 4 | | Myrtaceae | 4 | 2 |
| Annonaceae | 17 | 2 | Ochnaceae | 7 | 1 |
| Apocynaceae | 9 | | Octonemataceae | 1 | |
| Asteraceae | 2 | | Olacaceae | 12 | 1 |
| Bignoniaceae | 1 | | Opiliaceae | 2 | |
| Bombacaceae | 1 | | Pandaceae | 2 | |
| Boraginaceae | 1 | | Passiloraceae | 1 | |
| Burceraceae | 3 | | Polygalaceae | 1 | |
| Caesalpiniaceae | 20 | | Rhamnaceae | 2 | |
| Chailletiaceae | 1 | | Rubiaceae | 61 | 5 |
| Chrysobalanaceae | 10 | 2 | Rutaceae | 7 | |
| Clusiaceae | 13 | 1 | Sapindaceae | 15 | |
| Combretaceae | 2 | | Sapotaceae | 8 | 1 |
| Connaraceae | 2 | | Scytopetalaceae | 5 | |
| Ebenaceae | 13 | | Sterculiaceae | 25 | 1 |
| Erythroxylaceae | 1 | | Thymeliaceae | 1 | |
| Euphorbiaceae | 32 | 5 | Tiliaceae | 3 | |
| Fabaceae | 5 | | Verbenaceae | 3 | |
| Flacourtiaceae | 9 | 3 | Violaceae | 12 | 1 |
| Hippocrateaceae | 3 | | Vochysiaceae | | 2 |
| Icacinaceae | 2 | 1 | | | |
| Irvingiaceae | 5 | 1 | Total | 400 | 36 |
| Lauraceae | 7 | 1 | | | |
| Lecytidaceae | 3 | | | | |
| Lepidobotryaceae | 1 | | | | |
| Liliaceae | 4 | | | | |
| Longaniaceae | 3 | | | | |
| Medusandraceae | 1 | | | | |
| Melastomataceae | 10 | 2 | | | |
| Meliaceae | 8 | 3 | | | |
| Mimosaceae | 6 | | | | |
| Monimiaceae | 1 | | | | |

**Table 5 Plant species producing positive results**

| code (original) | results of first test: mating 12 h | results of first test: two hybrid 18h | code (positives) | results of confirmatory test: mating a | mating b | two hybrid | two hybrid | Classification |
|---|---|---|---|---|---|---|---|---|
| P24A-7 | + | - | W25 | ++ | ++ | - | - | Rincrea lepidobotrys |
| P24A-14 | + | - | W23 | + | ++ | - | - | Klaineanthus gaboniea |
| P24A-25 | ++ | - | W19 | ++ | ++ | - | - | Drypetes Molunduana |
| P24B-6 | ++ | - | W17 | + | + | - | - | Syzigium roulandii |
| P24B-12 | ++ | - | W16 | + | + | - | - | Leptaulus daphnoides |
| P24B-18 | - | +- | W15 | + | +- | +- | +- | Homalium letestui |
| P24B-19 | - | + | W14 | + | + | +- | +- | Sapium |
| PR3-2 | - | +++ | W35 | +- | +- | +++ | +++ | Trichilia sp (TRSP) |
| EA-32 | ++ | - | W39 | + | +- | - | - | Anthronotha fragrans |
| EB-12 | +++ | - | W40 | + | - | - | - | Aframomun sp |
| EC-6 | ++ | - | W44 | ++ | + | - | - | fern |
| EC-10 | - | ++ | W45 | - | - | ++ | + | Casearia sp (WHOM) |
| EC-32 | + | - | W48 | - | +- | - | - | Musuondia sp |
| KB-27 | +++ | - | W56 | + | + | - | | Klainedoxa gabonensis |
| KB-29 | +++ | - | W57 | ++ | ++ | - | | unknown |
| PR4-1 | + | - | W58 | ++ | ++ | - | | Memecylon Englerianum |
| PR4-2 | ++ | - | W59 | + | + | - | | Engomegoma gordonii |
| PR4-8 | + | - | W60 | + | ++ | - | | Homalium sarcopetallum |
| PR4-13 | ++ | - | W61 | ++ | ++ | - | | Cordia sp |
| PR4-18 | + | - | W62 | + | + | - | | MARA |
| PR4-19 | + | - | W63 | + | + | - | | Beilshmieda sp |
| PR4-22 | + | - | W65 | ++ | ++ | - | | Dorstenia turbinata |
| PR4-23 | ++ | - | W66 | + | + | - | | ACAN |
| PR4-33 | ++ | - | W67 | ++ | ++ | +/- | | Brenania brieyi |
| PR3-21 | + | - | W69 | (+) | (+) | - | | Uvariopsis ellipticum |
| P24B-23 | - | + | W12 | ++ | ++ | - | - | Uvariopsis bakeriana |

**Table 5 continued**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| P24B-25 | + | - | W11 | + | +- | - | - | SOYA |
| P24B-26 | + | - | W13 | + | + | - | - | Xylopia (?) |
| P24C-13 | ++ | - | W7 | + | ++ | - | - | Ouratea vogelii |
| P24C-21 | + | - | W6 | ++ | ++ | - | - | Schamaniophyton magnificum |
| P24C-31 | + | - | W4 | + | + | - | - | Uvariopsis Korupensis |
| P24C-33 | ++ | + | W3 | ++ | ++ | - | - | Hesteria parviflora |
| P23A-24 | - | + | W10 | + | ++ | ++ | ++ | Casearia sp (WHOM) |
| P23B-25 | + | - | W21 | + | ++ | - | - | Trichoscypha abut congolana |
| P23B-28 | + | - | W24 | + | ++ | - | - | Macaranga monandra |
| P22A-17 | ++ | - | W8 | +++ | +++ | - | - | Garcinia ovalifolia |
| P22A-18 | ++ | - | W9 | + | - | - | - | Eugenia talbotii |
| P21A-3 | + | - | W5 | ++ | ++ | - | - | Nauclea diderrichii |
| P21A-6 | - | +- | W29 | ++ | + | +- | +- | MOKO |
| P21A-18 | + | - | W26 | ++ | ++ | +- | - | Turreanthus mannii |
| P21A-21 | - | ++ | W30 | - | +- | + | + | RUDO |
| PR1-2 | + | - | W31 | ++ | ++ | - | - | Cola sp (COBE) |
| PR1-11 | + | - | W32 | ++ | ++ | - | - | Synsepullum stipulatum |
| PR1-27 | + | - | W34 | +++ | + | - | - | Musaenda sp |
| PH1-30 | + | - | W1 | ++ | ++ | - | - | Nephthytis sp |
| PH2-3 | - | + | W18 | +++ | +++ | + | ++ | Bulbophylum |
| PH2-24 | + | - | W27 | + | + | - | - | Scyphosyce manniana |
| PR2-3 | + | - | W2 | ++ | ++ | - | - | Bridelia micrantha |

Whenever possible, the genus and species of plants testing positive by the two bioassays is indicated in the table above. Plus marks (+) indicate a positive result for either the mating test, or the two-hybrid dimerization assay. Three plus makes (+++) indicate a very streng positive result (extensive growth of the yeast indicator strain).

## Table 6

**Activity of plant extracts/isolated compounds vs. growth of African trypanosomes *in vitro*.** Bloodform trypanosomes were grown in 24 well culture dishes (1 ml/well) in HMI-18 medium (Hirumi & Hirumi 1989). One half of the culture volume was replaced daily with fresh medium plus drug. Each extract was dissolved in 100% DMSO and diluted with medium. Cells were counted daily with a coulter counter. Data are as $IC_{50}$ values in μg extract/ml culture. Four strains were used: *T. b. brucei* Lab 110 EATRO, and three *T. b. rhodesiense* clinical isolates from the Kenya Trypanosomiasis Research Institute (KETRI). All data from 48 hr cultures. Control cell counts averaged 5 x $10^6$ cells/ml at 48 h. (Data thru April 30, 2000).

| | | $IC_{50}$ (μg/ml) EATRO 110 | KETRI 243 | KETRI 269 | KETRI 243 As10-3 |
|---|---|---|---|---|---|
| *Mezoneurum benthamianum* | SU-1749 | 44 | 19.5 | 18.5 | 37 |
| *Mezoneurum benthamianum* | SU-1750 | 19 | 76 | 37 | 31 |
| *Eupatorium odoratum* sakuranetin** | SU-1751 | 20 | 20.5 | 73 | 20.5 |
| *Gnetum africanum* | SU-1752 | 202 | 190 | 225 | 200 |
| *Cryptolepis sanguinolenta* CRYPTOLEPINE** | SU-1754* | 0.008 | 0.09 | 0.0074 | 0.019 |
| *Plantex vellous* | SU-1756 | 75 | 18.5 | 13.5 | 6.6 |
| *Plantex vellous* | SU-1757 | 1.5 | 21.5 | 13 | 22 |
| *Fagara lemaire* | SU-1758 | 2.2 | 2.0 | 2.05 | 1.55 |
| *Fagara lemaire* | SU-1759 | 20.5 | 170 | 130 | 140 |
| *Erythrina senegalensis* | SU-1760 | 7.2 | 9.1 | 15.5 | 14.75 |
| *Erythrina senegalensis* | SU-1761 | 18.9 | 20 | 22 | 20.5 |
| *Mitracarpus scaber* | SU-1762 | 98 | 105 | 71 | 26 |
| *Olax viride* | SU-1763 | 195 | 32% @ 500 μg/ml | 235 | 300 |
| *Chasmanthera dependens* | SU-1764 | 225 | 225 | 225 | 220 |
| *Dracaena mannii* *Mannispirostan A* ** | SU-1765 | 200 | 200 | 200 | 180 |
| *Glossocalyx brevipes* | SU-1768 | 0.78 | 0.76 | 0.715 | 1.3 |
| *Dorsternia barteri* | SU-1769 | 7.5 | 7.3 | 15.25 | 6.1 |
| *Dorsternia barteri* | SU-1770 | 16.5 | 19.25 | 16.0 | 6.8 |
| *Gnetum africanum* | SU-1771 | 54 | 60 | 56 | 29.5 |
| *Gnetum africanum* | SU-1772 | 50 | 47 | 35.5 | 17.5 |
| *Garcinia kola* | SU-1773 | 210 | 210 | 210 | 180 |

*diluted with 0.1 M Tris-saline pH 7.4

**pure compounds see structure in figure

**Table 7**

**$IC_{50}$ values for Isolated Compounds** were tested vs. trypanosome isolates grown in bloodforms in HMI-18 medium containing 20% fetal bovine serum earlier. Coulter counts were made daily and $IC_{50}$ values determined after 48 h. (Data thru April 30, 2000).

| | | | KETRI | | |
|---|---|---|---|---|---|
| | | **Lab110 EATRO** | **243** | **269** | **243 As 10-3** |
| *Aframomum aulacocarpus*** AZ2 | SU-1460 A | 0.59 | 0.18 | 0.62 | 0.59 |
| *Glossocalyx brevipes* | S-1464 A | 0.7 | 0.088 | 0.29 | 0.195 |
| *Glossocalyx brevipes* | S-1464 B | 0.235 | 0.18 | 0.155 | 0.335 |
| | DC-1 | 7.2 | 0.7 | 1.1 | 17 |
| | DC-2 | 7.0 | 1.95 | 1.25 | 2.05 |
| | PH-1 | 5.9 | 14.5 | 20 | 48 |
| | PH-2 | 2.3 | 11 | 9.4 | 13 |
| | XBX-2 | 1.49 | 3.9 | 5.6 | 2.05 |
| *Aframomum letestuianum* | AL+6 | 1.4 | 2.35 | - | - |
| *Aframomum letestuianum* | AL+7 | 67 | 115 | - | - |
| *Aframomum letestuianum* | AL+10 | 2.65 | 2.8 | | |
| *Discoglypremna coloneura* | ND1 | 3.2 | 6.o | - | - |
| *Discoglypremna coloneura* | ND2 | 11.5 | 7.9 | - | - |
| *Discoglypremna coloneura* | ND3 | 22%@500 μg/ml | 22%@500 μg/ml | | |
| *Discoglypremna coloneura* | ND4 | 19 | - | | |
| *Discoglypremna coloneura* | ND5 | 1.9 | - | - | - |
| Control | Pentamidine | 0.00048 | 0.00186 | 0.0019 2 | 0.003 |
| Control | Melarsen oxide | 0.00077 | 0.0025 | 0.0066 | 0.0072 |

**pure compounds see structure in figure

**Table 8 : Most active plant extracts for *in vivo* testing in trypanosome screen.**

WRAIR supplied:

SU-1754, 1757, 1758, 1760, 1761, 1768, 1769, 1770.

AP-2 supplied:

SU-1460A, 1460B, 1464A, 1464B, DC1, DC2, PH-1, Ph-2, XBX-2

**Table 12b : Summary of testing with ICBG compounds vs. *T. b. brucei* mouse model**

Groups of 3 mice are infected with 2.5 x $10^5$ trypanosomes and treatment is begun 24 h post-infection. Treatment is given once daily x 3 days, usually at 1, 5, 10, and 25 mg/kg, i.p.

All of the following were inactive at the dosages tested. They did not prolong life beyond that of the untreated controls:

- SU-1460A, SU-1460B
- AZ2 (SU-1460: up to 50 mg/kg x 3 days)

SU-1461 (up to 50 mg/kg i.p. and p.o.)

**Table 8b : Summary of testing anti-hypercholesteremia agents vs. *T. b. brucei* mouse model**

- Groups of 3 mice were infected with 2.5 x $10^5$ trypanosomes and treatment was begun 24 h post-infection.
- Pills were ground using a mortar and pestle, and the compounds were suspended in 2% methylcellulose containing 0.5% Tween 80.
- Agents were administered orally once daily for 3 days.
- Doses used were 25, 50, 100 mg/kg/day for Lamisil, Mevacor, Pravachol, Zocor, and Lescol.
- Lopoid was used at 200, 400, 600 mg/kg/day.
- Doses were calculated based on the percent active compound in each pill or capsule.
- None of the agents increased survival time beyond that of the infected untreated controls.
- Ketoconazole was also tested in this system at 15, 30, 45, and 60 mg/kg/day x 5 days, p.o. and i.p. dose regimens were used. None were effective.

**Table 9 Inhibition of Trichomonas growth by new primary plant extracts.** The assay system used was the standard MIC (minimal inhibitory concentration) assay (Meingassner et al 1978) for Trichomonas in which serial dilutions were prepared in medium using sterile 96 well plates. Twelve dilutions were made, with a concentration range of 2.5 to 0.0012 mg/ml. Each well contained $10^4$ organisms. Plates were incubated aerobically for 48h then examined. The MIC is defined as the minimum concentration of drug in which no motile organisms are visible after 48 h incubation. CI-NIH is metronidazole sensitive, CDC-085 is metronidazole-resistant and KV1 is the cattle parasite, *Tritrichomonas foetus*. Data expressed as MIC in mg/ml. ND, not determined.

| Origin | Extract | MIC | | |
|---|---|---|---|---|
| | | C1-NIH | CDC-085 | KV1 |
| *Araliopsis tabouensis*** AT7 | SU 1458 | 0.2 | 0.4 | 0.4 |
| *Aframonum aulocacarpus*** AZ2 | SU 1460 | 0.1 | 0.0015 | 0.1 |
| *Dracaena mannii Mannispirostan A* | SU 1461 | 0.0125 | 0.006 | 0.05 |
| *Napoleonaea imperialis* MEOH | SU 1462 | 0.1 | - | 0.4 |
| *Pachypodanthium staudtii* $CH_2Cl_2$ | SU 1463 | 0.80 | ND | >0.80 |
| *Glossocalyx brevipes* $CH_2Cl_2$ | SU 1464 | 0.0125 | 0.0125 | 0.0125 |
| *Enantia chlorantha* MeOH | SU 1465 | 0.80 | 0.10 | >0.80 |
| *Eupatorium odoratum* MEOH | SU 1466 | 0.4 | 0.4 | 0.4 |
| *Cleistopholis patens* EtOH | SU 1467 | >0.80 | 0.10 | >0.80 |
| *Leidobotrys staudii* $CH_2Cl_2$ | SU 1468 | 0.40 | ND | >0.80 |
| *Ancistrocladus bateri* ABSBM | SU 1469 | 0.40 | ND | 0.40 |
| *Eupatorium adoratum* | SU 1751 | 0.3 | 0.6 | - |
| *Eupatorium adoratum* MEOH | SU 1752 | 0.3 | 0.3 | - |
| *Fagara lemairei* | SU 1758 | 0.6 | - | - |
| *Fagara lemairei* MEOH | SU 1759 | 0.6 | - | - |
| *Mitracarpus scaber* (Pet. Ether) | Su 1762 | 0.1 | 0.9 | - |
| Control | Metronidazole | 0.003 | 0.40 | 0.003 |

**pure compounds see structure in figure

Table 10

# Antituberculosis Data

| Plant Name | | SU | L1 PI | L2 MIC | L2 IC50 | L2 Comment |
|---|---|---|---|---|---|---|
| Aframomum daniellii | Pet ether | 1423 | 23 | | | |
| Alchornea cordifolia | Alkaloid mix MeOH | 1424 | -5 | | | |
| Chasmanthera dependens | Quarter alkd | 1425 | 2 | | | |
| Chasmanthera dependens | Palmatine | 1426 | 90 | 100 | | MIC of RMP=0.06 |
| Chrysophyllum albidum | MeOH | 1427 | -10 | | | |
| *Napoleonaea imperialis* | MeOH saponin | 1428 | -15 | | | |
| *Napoleonaea imperialis* | Np series | 1429 | -7 | | | |
| Dorstenia multiradiata | MeOH | 1430 | 99 | | 9.85 | No MIC data yet |
| Crescentia cujete | CC1 | 1431 | 31 | | | |
| Crescentia cujete | mix | 1432 | 47 | | | |
| Dracaena mannii | Spirokanozole | 1433 | -7 | | | |
| Dracaena mannii | MeOH /lower layer | 1434 | -6 | | | |
| Enantia chlorantha | ENM7-14 | 1435 | 99 | 100 | | MIC of RMP=0.06 |
| Enantia chlorantha | Palmatine | 1436 | | | | |
| Garcinia kola GB1 | | 1437 | -3 | | | |
| Garcinia kola GB2 | | 1438 | -4 | | | |
| Hippocratea welwitschii | Alkaloid mixture | 1439 | -10 | | | |
| Eugenia uniflora | hexane | 1440 | 101 | 12.5 | 2.5 | MIC of RMP=0.06 |

## Fig 2-6 Phytomedicine: Sample of Certificate of Analysis used at InterCEDD

### CERTIFICATE OF ANALYSIS

**1. Product**: ***Aframomum melegueta*** Seeds

| **2. Attribute** | **Results** |
|---|---|
| Sample Batch | 99109 |
| Appearance | coarse Powder |
| Colour | dark brown |
| Taste | peppery/ spicy |
| Odour | characteristic |
| Heavy Metals | Tested for lead and cadmium: non detected for value of less than 0.1 mg/ kg. |
| B. Ash Values | within limits (see specifications) |
| Ethanol soluble extractive | within limits (see specifications) |
| Water soluble extractive | within limits (see specifications) |

**3. Chemical Assay/ Test for Identity**

| | |
|---|---|
| Essential oil: | values within limits and compared with reference standard. |

**4. Microbial Characteristics**

| | |
|---|---|
| Moulds & Yeast | Non detected |
| *Escherichia coli* | negative |
| *Salmonella spp* | negative |

**Microbiology:** The test for Salmonella spp in *Aframomum melegueta* aqueous extract was negative. The maximum acceptable limits for other microorganisms are within the recommended levels (*Quality control methods for medicinal plants. Geneva, World Health Organization, 1998.*) Aerobic bacteria- not more than $10^7$/g; fungi –not more than $10^5$/g; *Escherichia coli*- not more than $10^2$/g.

Signature of Supervising Scientist:
Date:

Fig 3

## CERTIFICATE OF ANALYSIS

**1. Product**: *Garcinia kola* Seeds

| **2. Attribute** | **Results** |
|---|---|
| Sample Batch | 99101 |
| Appearance | Coarse Powder |
| Colour | Off-white/ brown |
| Taste | bitter |
| Odour | characteristic |
| Heavy Metals | Tested for lead and cadmium: non detected for value of less than 0.1 mg/ kg. |
| C. Ash Values | Within limits (see specifications) |
| Ethanol soluble extractive | Within limits (see specifications) |
| Water soluble extractive | Within limits (see specifications) |

**4. Chemical Assay/ Test for Identity**

| | |
|---|---|
| Biflavonoids | HPLC and Capillary electrophoresis used for qualitative detection of marker substances, kolaviron (see attached chromatogram. |

**4. Microbial Characteristics**

| | |
|---|---|
| Moulds & Yeast | Non detected |
| *Escherichia coli* | negative |
| *Salmonella spp* | negative |

**Microbiology:** The test for Salmonella spp in *Garcinia kola* aqueous extract was negative. The maximum acceptable limits for other microorganisms are within the recommended levels (*Quality control methods for medicinal plants. Geneva, World Health Organization, 1998.*) Aerobic bacteria- not more than $10^7$/g; fungi –not more than $10^5$/g; *Escherichia coli*- not more than $10^2$/g.

Signature of Supervising Scientist:

Date:

Fig 4

## CERTIFICATE OF ANALYSIS

**1. Product**: ***Gongronema latifolia*** Leaves

| **2. Attribute** | **Results** |
|---|---|
| Sample Batch | 99102 |
| Appearance | Coarse Powder |
| Colour | dark brown |
| Taste | bitter |
| Odour | characteristic |
| Heavy Metals | Tested for lead and cadmium: non detected for value of less than 0.1 mg/ kg. |
| D. Ash Values | within limits (see specifications) |
| Ethanol soluble extractive | within limits (see specifications) |
| Water soluble extractive | within limits (see specifications) |

**5. Chemical Assay/ Test for Identity**

| | |
|---|---|
| Phytosterols: | Thin layer chromatographic analysis: finger printing using β-sitosterol as marker.. |

**4. Microbial Characteristics**

| | |
|---|---|
| Moulds & Yeast | Non detected |
| *Escherichia coli* | negative |
| *Salmonella spp* | negative |

**Microbiology:** The test for Salmonella spp in *Gongronema latifolia* aqueous extract was negative. The maximum acceptable limits for other microorganisms are within the recommended levels (*Quality control methods for medicinal plants. Geneva, World Health Organization, 1998.*) Aerobic bacteria- not more than $10^7$/g; fungi –not more than $10^5$/g; *Escherichia coli*- not more than $10^2$/g.

Signature of Supervising Scientist:

Date:

Fig 5

## CERTIFICATE OF ANALYSIS

**1. Product**: ***Ocimum gratissimum*** leaves

| **2. Attribute** | **Results** |
|---|---|
| Sample Batch | 99106 |
| Appearance | coarse powder |
| Colour | dark brown |
| Taste | aomatic |
| Odour | characteristic |
| Heavy Metals | Tested for lead and cadmium: non detected for value of less than 0.1 mg/ kg. |
| E. Ash Values | within limits (see specifications) |
| Ethanol soluble extractive | within limits (see specifications) |
| Water soluble extractive | within limits (see specifications) |

**6. Chemical Assay/ Test for Identity**

| | |
|---|---|
| Essential oil: | values within limits and compared with reference standard. |

**4. Microbial Characteristics**

| | |
|---|---|
| Moulds & Yeast | Non detected |
| *Escherichia coli* | negative |
| *Salmonella spp* | negative |

**Microbiology:** The test for Salmonella spp in *Ocimum gratissimum* aqueous extract was negative. The maximum acceptable limits for other microorganisms are within the recommended levels (*Quality control methods for medicinal plants. Geneva, World Health Organization, 1998.*) Aerobic bacteria- not more than $10^7$/g; fungi –not more than $10^5$/g; *Escherichia coli-* not more than $10^2$/g.

Signature of Supervising Scientist:
Date:

**Fig 6**

## CERTIFICATE OF ANALYSIS

**1. Product**: ***Zingiber officinale*** Rhizome

| **2. Attribute** | **Results** |
|---|---|
| Sample Batch | 99108 |
| Appearance | coarse Powder |
| Colour | dark brown |
| Taste | spicy |
| Odour | characteristic |
| Heavy Metals | Tested for lead and cadmium: non detected for value of less than 0.1 mg/ kg. |
| F. Ash Values | within limits (see specifications) |
| Ethanol soluble extractive | within limits (see specifications) |
| Water soluble extractive | within limits (see specifications) |

**7. Chemical Assay/ Test for Identity**

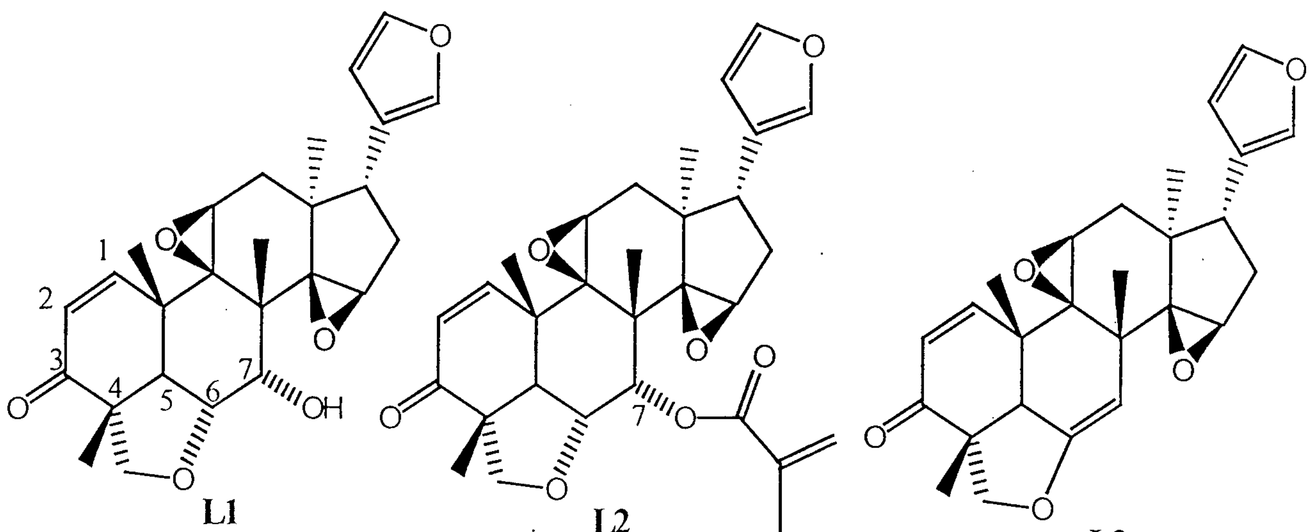
| | |
|---|---|
| Essential oil: | values within limits and compared with reference standard. |

**4. Microbial Characteristics**

| | |
|---|---|
| Moulds & Yeast | Non detected |
| *Escherichia coli* | negative |
| *Salmonella spp* | negative |

**Microbiology:** The test for Salmonella spp in *Zingiber Officinale* aqueous extract was negative. The maximum acceptable limits for other microorganisms are within the recommended levels (*Quality control methods for medicinal plants. Geneva, World Health Organization, 1998.*) Aerobic bacteria- not more than $10^7$/g; fungi –not more than $10^5$/g; *Escherichia coli*- not more than $10^2$/g.

Signature of Supervising Scientist:
Date:




L1 L2 L3

Figure 7 Structures of Three Novel Limonoids (L1-L3) Active against Cystic Fibrosis